BIOCELL
2008, 32(1): 41-47

ISSN 0327 - 9545
PRINTED IN ARGENTINA

Orbicules diversity in *Oxalis* species from the province of Buenos Aires (Argentina)

Sonia Rosenfeldt and Beatriz Gloria Galati

Departamento de Biodiversidad y Biología Experimental. Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires. 4º piso Pabellón II. Ciudad Universitaria. C1428EHA. Nuñez. Ciudad Autónoma de Buenos Aires. Argentina.

Key words: embryology, orbicules, *Oxalis*, taxonomy, pollen.

ABSTRACT: Eleven *Oxalis* L. species from the province of Buenos Aires (Argentina) were investigated with scanning and transmission electron microscopes. We identified four different types and two subtypes of orbicules. We conclude that the close morphological similarity between these species is also reflected in their orbicules, and we suggest that the orbicules morphology may be a useful character in systematic studies.

Introduction

Ubisch (1927) observed granular bodies along the inner tangential walls of the tapetal cells in *Oxalis rosea,* and she pointed out the similarities of these bodies to pollen exine in staining properties. However, until now, ultrastructural and ontegenetic studies of these bodies have been completed for only three species of this genus (Carniel, 1967; Rosenfeldt and Galati, 2005).

Kosmath (1927) studied the presence of orbicules in 69 taxa from 35 families. Later, Bhandari and Kishori (1971) considered that the orbicules are composed of sporopollenin because the pollen exine and Ubisch bodies have a specific staining pattern with spirit-soluble aniline blue.

Heslop-Harrison (1968) named these bodies "orbicules", but Bhandari (1984) preferes the term Ubisch bodies because it has been long used in the literature and is well understood both by LM and EM embryologists.

The orbicules can be defined as corpuscules of variable size (0.14 μm-20 μm) that show the same electron density, autofluorescence, resistance to acetolysis, and reaction to the dyes as pollen exine.

The parallelism between the pollen exine and the orbicule wall is explained by Hess (1985, 1986) as rooted in the homology of tapetum and sporogenous tissue. Because ornamentation of the pollen exine offers useful characters for systematics, orbicules might also have taxonomic value.

The taxonomic usefulness of orbicule characters has so far only been studied in few angiosperm taxa: the Chloanthaceae, now included in Lamiaceae (Raj and El-Ghazaly, 1987); *Euphorbia* L. (El-Ghazaly, 1989; El-Ghazaly and Chaudhary, 1993); the subfamily Cinchonoideae of Rubiaceae (Huysmans *et al*., 1997); the order Gentianales (Vinckier *et al*., 2000; Vinckier and Smets, 2002 a, b, c, 2003); and the genera *Dioscorea* L. (Schols *et al*., 2001).

The aim of this study was 1) to provide detailed orbicules morphological and ultrastructural descriptions

Address correspondence to: Dra. Sonia Rosenfeldt. Depto. de Biodiversidad y Biología Experimental. Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires. Ciudad Universitaria, Pabellón II, 4º piso. C1428EHA Nuñez. Ciudad Autónoma de Buenos Aires, ARGENTINA.
E-mail: *soniar@bg.fcen.uba.ar / galati@bg.fcen.uba.ar*
Received on May 31, 2007. Accepted on September 23, 2007.

in 11 species of *Oxalis* with the use of SEM and TEM and 2) to explore the possibilities of employing these characters in taxonomic considerations.

Materials and methods

This study was based on materials from 11 species and two varieties of *Oxalis* collected from the province Buenos Aires and the city of Buenos Aires, Argentina.

Specimens studied are listed in alphabetical order:
O articulata Savigny 27974 (SI); *O. conorrhiza* Jacqui 2075 (SI);
O. corniculata L. var *atropurpurea* Planchon 43177 (CTES); *O. corniculata* L. var *corniculata* 555 (SI); *O. debilis* Humboldt, Bonpland et Kunth 2015 (SI);
O. floribunda Lehmann 28745 (SI); *O. hispidula* Zuccarini 1345 (SI);
O.lasiopetala Zuccarini 1673 (SI); *O. niederleinii* Knuth 1042 (SI);
O. paludosa Saint Hilaire 1764 (SI); *O. perdicaria* Bertero 20045 (SI);
O. refracta Saint Hilaire 28695 (SI).

For Scanning Electron Microscopy (SEM) studies, the anthers were fixed in FAA (formalin, alcohol, acetic acid), were transferred to 100% acetone and were air dried. The sputtering treatment was made with gold-palladium for 3 min. Scanning micrographs were taken with a Philips XL 30 microscope.

For Transmission Electron Microscopy (TEM) studies, the anthers were pre-fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.2) for 2 h and then post-fixed in OsO_4 at 2ºC in the same buffer for 3 h. Following dehydration in ethanol series, the material was embedded in Spurr's resin. Fine sections were made on a Sorvall ultramicrotome and then stained with uranyl acetate and lead citrate (O'Brien and Mc Cully, 1981). The sections were observed and photographed with a JEOL - JEM 1200 EX II TEM at 85.0 Kv.

Results

Orbicules characters

A) Morphology

The orbicules are randomly dispersed on the inner surface of the anther locule. They are plate-like and more or less rounded in most of the species studied (Figs.1A, C, E, G; 2A, C, E and 3F).

The surface of the orbicules is generally smooth (Figs. 1A, C, E and 2A, C). In a few cases, the surface of orbicules has minute verrucoid processes (Figs. 3C, D). Perforations are found in some species (Figs. 1F, G and 2C, E).

There is also considerable variation in the size of orbicules (1-13 μm), even within a single species.

In some cases, the orbicules fuse their corpus, which results in aggregates of two or more orbicules that are observed as rod-like to elongated units with a lightly wavy margin (Figs. 3A, C).

B) Ultrastructure

The orbicules of the species studied have one to several central cores and a corpus or wall that is similar to the ektexine in electron-density (Figs. 1B, D, H; 2B, D, F and 3B, E, G). The central core has the appearance of a vesicle, transparent to the electrons in medium section (Figs. 1B, D, H; 2B, D, F and 3B, E, G). Its diameter varying between 0.28 μm and 0.8 μm. In some species, the central core is in contact with the inner loculus surface (Figs. 1B and 2B, D, F) and the perforations end in the surface of the central core (arrows) (Figs. 1H and 2D).

In some species, the surface of the orbicule that is in contact with the microsporangium wall presents irregular invaginations (arrow heads) (Figs. 2D, F; 3B, E, G).

A very electron-dense peripheral layer, which delimits the homogeneous electron-dense orbicule wall, is present in all the species studied (Figs. 1B, D; 2B, D and 3B, E, G). This layer is less evident when the orbicules are not fully mature (Figs.1H; 2D, F).

Orbicules types (Table):

Type I: The orbicules are plate-like, more or less rounded, rarely irregular in form, and with a smooth surface. Taxa included: *O articulata* Savigny: *O.lasiopetala* Zuccarini, *O. floribunda* Lehmann (Figs. 1A-E).

Type II: The orbicules are plate-like, with smooth surface. They may have no or one perforation. When a single perforation is present, the orbicules are doughnut shaped. The orbicules fuse resulting in aggregates with two or three perforations. Taxa included: *O. paludosa* Saint Hilaire, *O. conorrhiza* Jacquin, *O .corniculata* L. var *atropurpurea* Planchon and *O. corniculata* L. var *corniculata* (Figs. 2A-F).

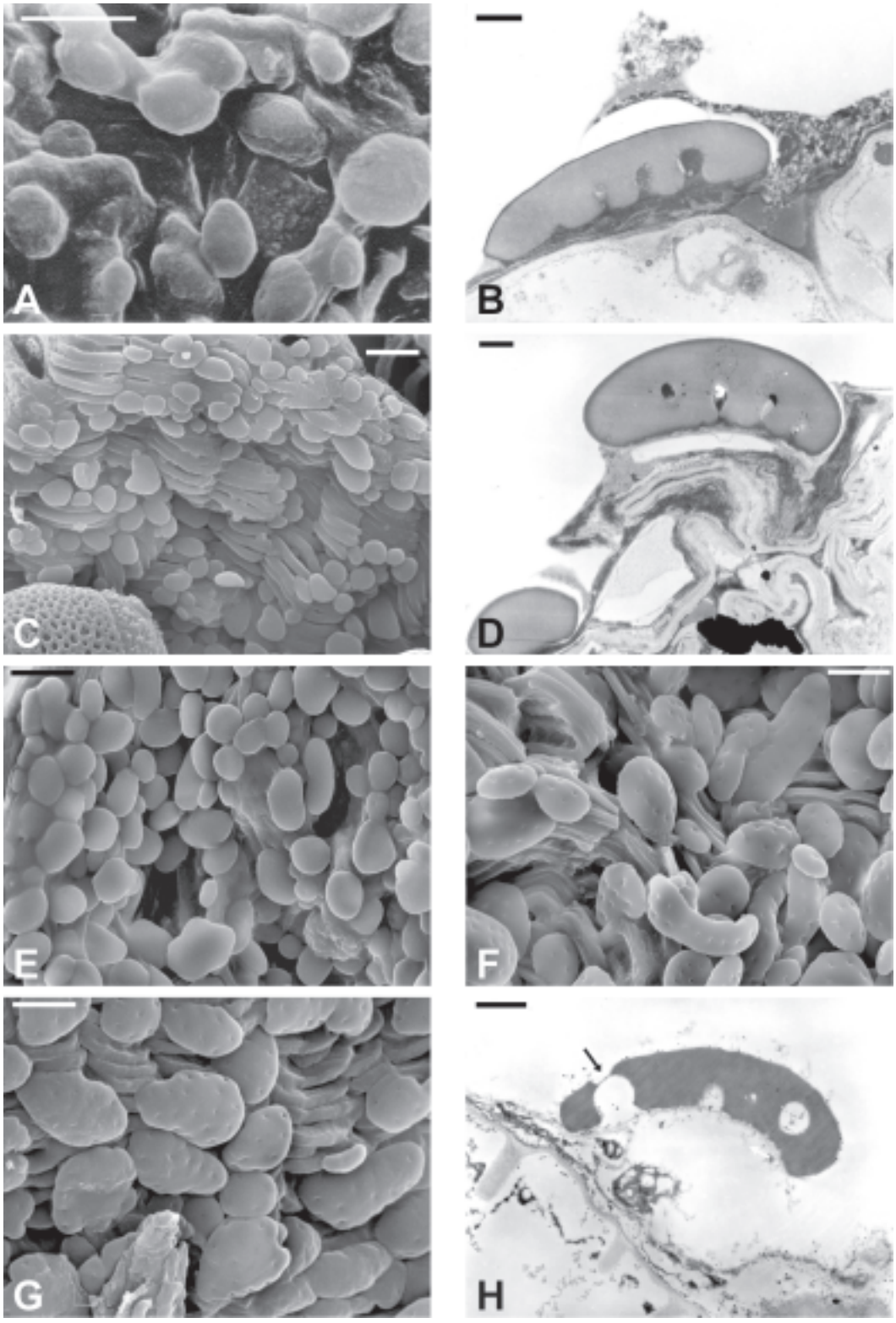

FIGURE 1. Orbicules morphology (A, C, E, F, G) and ultrastructure (B, D, H) of *Oxalis* spp. Types I and IV. (A-B) *O. articulata*. (C-D) *O. lasiopetala.* (E) *O. floribunda.* (F) *O. refracta.* (G-H) *O. niederleinii.* Arrow showing the perforation end in the surface of the central core. Scale Bars = 5μm (A, E, F), 4μm (C, G) 1μm (H) and 0,5μm (B, D).

Type III: The orbicules are plate-like and irregular in shape. The orbicules are fused, resulting in rod-like aggregates. The central cores are small (0.28-0.33 μm) (Fig. 3E, arrow head) and, thus, cannot be observed with a light microscope.

Subtype III A: The surface of the orbicules is smooth to slightly verrucose and the margin is straight. Taxa included: *O. hispidula* Zuccarini, *O. perdicaria* Bertero (Figs. 3C-G).

Subtype III B: The surface of the orbicules is smooth and the margin is wavy. Occasional perforations are present. Taxa included: *O.debilis* Humboldt, Bonpland et Kunth (Figs. 3A, B).

Type IV: The orbicules are plate-like and have a irregular contour, a smooth surface, and numerous perforations. Taxa included: *O. niederleinii* Knuth and *O. refracta* Saint Hilaire (Figs. 1F-H).

Discussion

The orbicules of the species of *Oxalis* L. studied are morphologically diverse in size and shape. Variation in the size of the orbicules has previously been observed in several species of *Euphorbia* (El-Ghazaly, 1989; El-Ghazaly and Chaudhary, 1993) and in species of Cinchonoideae (Rubiaceae) (Huysmans *et al*., 1997). According to Rowley and Walles (1985, 1987) and El - Ghazaly and Nilsson (1991), this variation in the size of the orbicules is due to the cycle of hyperactivity in tapetal cells during their development.

No intraspecific variation was observed, and all the specimens of the same species had the same orbicule morphology.

The general morphology of the *Oxalis* orbicules is plate-like with a smooth surface. The orbicules have one to several central cores, but multiple cores are not the result of the aggregate of several orbicules. In some species, several plate-like orbicules are aggregated resulting in orbicules that are rod-like or elongated units with a lightly wavy margin. All these characteristics define a new type of orbicules that can be added to Galati's classification (2003).

We observed an electron-dense peripheral sheet that covered the whole orbicular surface. According to Clément and Audran (1993a, b, c), this sheet can be found in each stage of orbicule development. However, in the *Oxalis* species that we studied, the sheet was not yet developed when the orbicule was not fully mature.

TABLE 1.

Orbicules Types.

Orbicule type		Morphology					Ultrastructure	Taxa	Section (Lourteig 2000)	Figures
		Form	Contour	Surface	Perforations	Aggregates	Central core Ø			
I		rounded plate-like irregular	rarely	smooth	-	-	0.25-0.5 μm	*O. articulata* Savigny, *O. lasiopetala* Zuccarini, *O. floribunda* Lehmann	Articulatae	1A-B 1C-D 1E
II		plate-like or doughnut shaped	rounded	smooth	none or one	Yes	0.30-0.80 μm	*O. paludosa* Saint Hilaire, *O. conorrhiza* Jacquin, *O. corniculata* L. var. *corniculata* and var. *atropurpurea* Planchon	Corniculatae	3A-B 3C-D 3F 3E
III	Sub type A	plate-like	irregular	smooth to lightly verrucose surface	-	Yes, rod like.	0.30-0.35 μm	*O. hispidula* Zuccarini, *O. perdicaria* Bertero	Ionoxalis	2C-E 2F-G
	Sub type B			smooth	occasional		0.25-0.30 μm	*O. debilis* Humboldt, Bonpland et Kunth.		2A-B
IV		Plate like	irregular	smooth	numerous	-	0.50-0.65 μm	*O. niederleinii* Knuth, *O. refracta* Saint Hilaire	Corniculatae	1G-H 1F

Oxalis orbicules can be grouped into four types and two subtypes according to their external and internal morphology. These types correspond with floral and vegetative characters used to separate species into different sections (Lourteig, 1983, 2000; Cabrera, 1965). The systematic value of the orbicule types was tested by comparing it to the most recent classification of the genus *Oxalis* (Lourteig, 2000).

Type I orbicules: *O. lasiopetala*, *O. floribunda* and *O. articulata* belong to the section Articulatae Knuth and are characterized by a cymose inflorescence with numerous flowers, a pink corolla, and an articulate rhizome.

Type II orbicules: *O. conorrhiza, O. corniculata* and *O. paludosa* belong to the section Corniculatae D.C. and have cymose inflorescence with one to three flower and a yellow corolla.

Type III orbicules: *O. hispidula*, *O. perdicaria* and *O. debilis* belong to the section Ionoxalis (Small) Knuth. All these species have bulbs. The first two species are

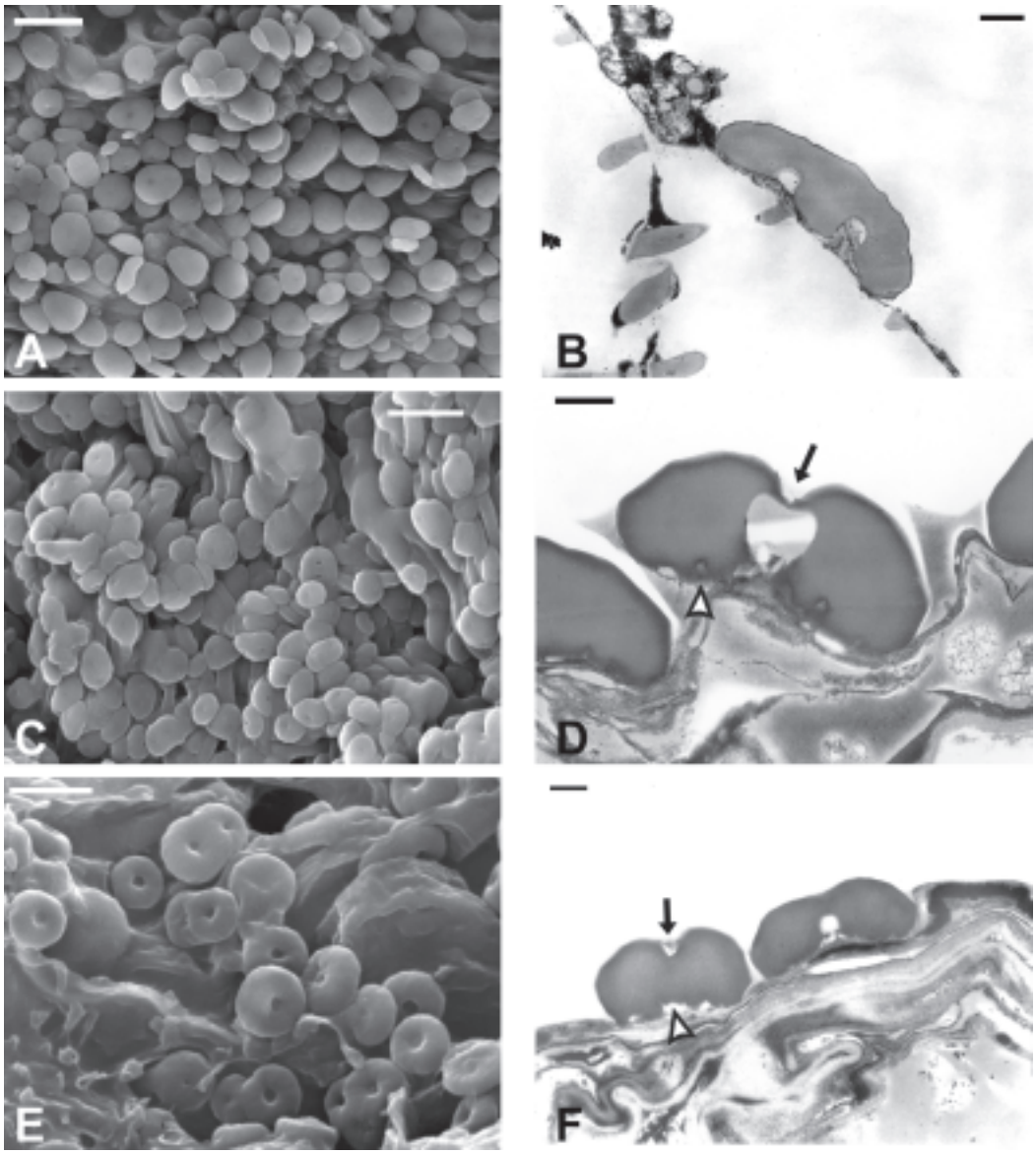

FIGURE 2. Orbicules morphology (A, C, E) and ultrastructure (B, D, F) of *Oxalis* spp. Type II. (A-B) *O. paludosa.* (C-D) *O. conorrhiza*. (E) *O. corniculata* var *atropurpurea*. (F) *O. corniculata* var *corniculata*. Arrow showing the perforation and arrow head showing irregular invaginations in contact with the microsporangium wall. Scale Bars = 5μm (A), 4μm (C), 2μm (E), 1μm (B), 0,5μm (D, F).

grouped in orbicules subtype A and have a cymose inflorescence with one to three flowers. In contrast, *O. debilis* has a cymose inflorescence with numerous flowers and a bulb with bulbils.

Type IV orbicules: *O. niederleinii* and *O. refracta* belong to the section Corniculatae D.C, have a cymose inflorescence with numerous flowers, a yellow corolla, and very branched stem with notable glandular hairs.

In a previous study about the pollen morphology in the same species of *Oxalis* (Rosenfeldt and Galati, 2007), four pollen types were described as follows:

Type I: granules assembled in clusters irregularly scattered on the colpus membrane. Taxa included: *O. articulata*, *O. lasiopetala*, and *O. floribunda.*

Type II: colpus membrane with irregularly scattered granules.

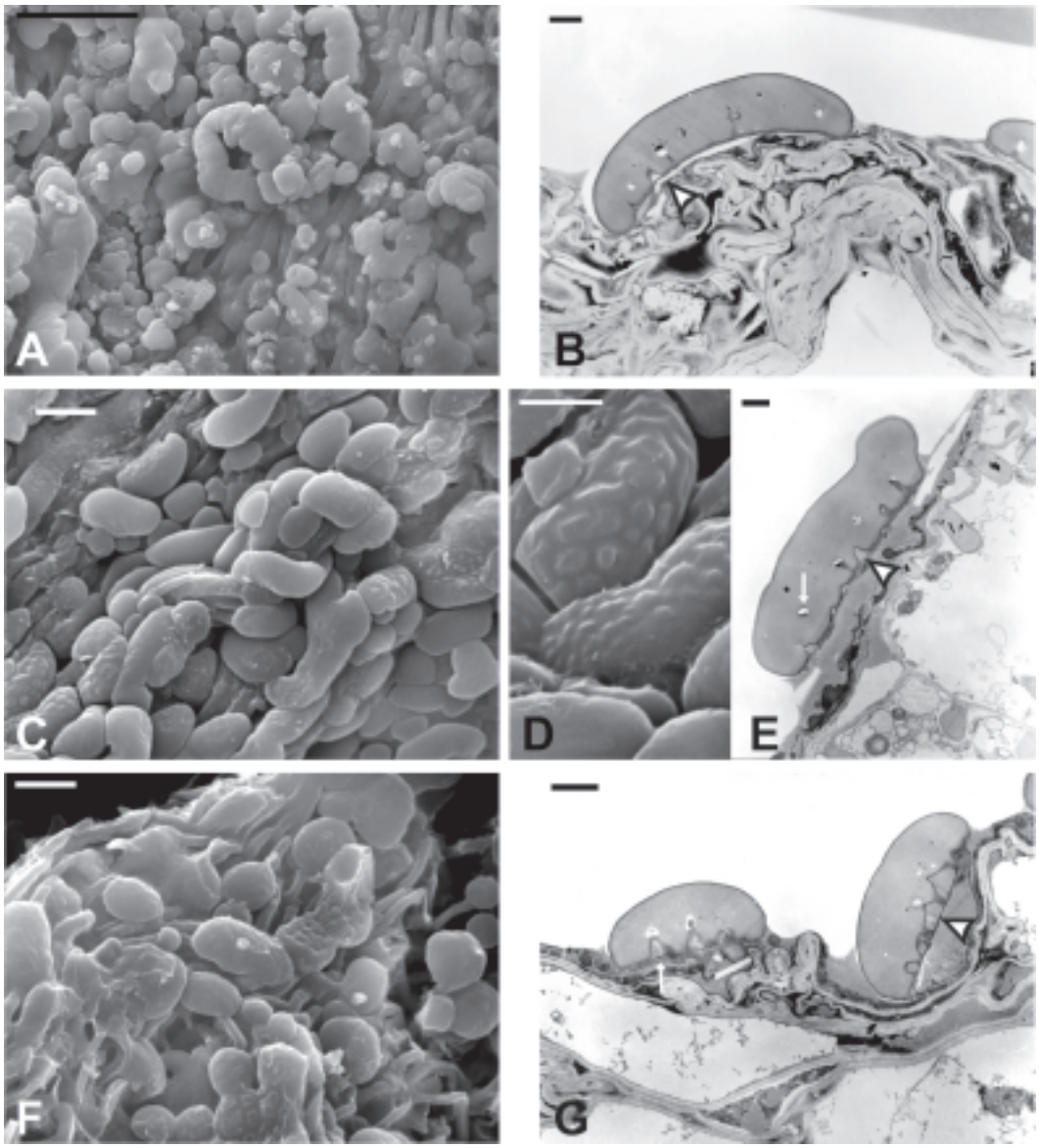

FIGURE 3. Orbicules morphology (A, C, D, F) and ultrastructure (B, E, G) of *Oxalis* spp. Type III. (A-B) *O. debilis*. (C-E) *O. hispidula.* (F-G) *O. perdicaria*. Arrow showing the central core and arrow heads showing irregular invaginations in contact with microsporangium wall. Scale Bars = 10 μm (A), 4μm (C, F), 2 μm (D) and 1μm (B, E, G).

Taxa included: *O. paludosa*, *O. conorhiza* and *O. corniculata.*
Type III: granules assamble in small clusters more or less aligned on the colpus membrane center. Taxa included: *O. hispidula*, *O. perdicaria* and *O.debilis.*
Type IV: granules assembled in comspicuous clusters extensively aligned on the colpus membrane center. Taxa included: *O. niederleinii.*

Strong correlations between pollen exine and orbicule surface ornamentation within the same species have been recorded for other genera (Huysmans *et al*., 1997; Vinckier *et al*., 2000; Vinckier and Smets, 2002a, b). However, in the *Oxalis* species we studied, the ornamentation of the pollen exine and that of the orbicule wall do not show striking analogies. Nevertheless, the *Oxalis* species belonging to each pollen type coincide with the species belonging to each orbicule type.

We conclude that the close morphological and palynological similarity between species is also reflected in their orbicules, and therefore the orbicule morphology can be used in the taxonomy of the genus *Oxalis*.

Acknowlegements

We thank Dr. Lara Strittmatter for her critical reading of the manuscript and Lic. Marina M. Gotelli for reviewing the English. This work was supported by Grant Nº 2514 from CONICET, Argentina.

References

Bhandari NN (1984). The microsporangium. *In:* Embryology of Angiosperms. B.M. Johri, Ed., Springer-Verlag, Berlin, pp. 53-111.

Bhandari NN, Kishori R (1971). Übisch granules on tapetal membranes in anthers; rapid selective staining by spirit blue. Stain Techn. 46: 15-17.

Cabrera AL (1965). Flora de la Provincia de Buenos Aires. Parte IV: Oxalidaceas- Umbeliferas. Colección Cientifica del I.N.T.A. Buenos Aires. Argentina.

Carniel K (1967). Licht- und electromenmikroskopische Untersuchung der UbischKörperentwicklung in der gattung *Oxalis*. Österr Bot Z. 114: 490-501.

Clément C, Audran JC (1993a). Cytochemical and ultrastructural evolution of orbicules in *Lilium*. Plant Syst Evol. 7: 63-74.

Clément C, Audran JC (1993b). Orbicule wall surface characteristic in *Lilium* (Liliaceae). Grana 32: 348-353.

Clément C, Audran JC (1993c). Electron microscope evidence for membrane around the core of Ubisch body in *Lilium* (Liliaceae). Grana 32: 311-314.

El-Ghazaly G (1989). Pollen and orbicule morphology of some *Euphorbia* species. Grana 28: 243-259.

El-Ghazaly G, Chaudhary R (1993). Morphology and taxonomic application of orbicules (Ubisch bodies) in genus *Euphorbia*. Grana Suppl. 2: 26-32.

El-Ghazaly G, Nilsson S (1991). Development of tapetum and orbicules of *Catharantus roseus* (Apocynaceae). *In:* Pollen Spores. S.Blackmore and S.H.Barnes, Eds., Syst. Ass. Special Vol. 44. Clarendon Press, Oxford, pp. 317-329.

Galati BG (2003). Ubisch bodies in Angiosperms. *In:* Advances in Plant Reproductive Biology, Vol. II. A.K. Pandey and M.R. Dhakal, Eds., Narendra Publishing House, Delhi, pp. 1-21.

Heslop-Harrison J (1968). Tapetal origin of pollen coat substances in *Lilium.* New Phytol. 67: 779-786.

Hess M (1985). Hemispheric surface processes of exine and orbicules in *Calluna* (Ericaceae). Grana 24: 93-98.

Hess M (1986). Orbicules and the ektexine are homologous sporopollenin concretions in Spermatophyta. Plant Syst Evol. 153: 37-48.

Huysmans S, El-Ghazaly G, Nilsson S, Smets E (1997). Systematic value of tapetal orbicules: a preliminary survey of the Cinchonoideae (Rubiaceae). Can J Bot. 75: 815-826.

Kosmath L. Von. (1927). Studien über das antherentapetum. Österr Bot. Z 76: 235-241.

Lourteig A (1983). Flora Ilustrada Caterinense. Fascículo: Oxalidaceas. Iajaí-Santa Catarina. Brasil.

Lourteig A (2000). *Oxalis L*. subgéneros *Monoxalis* (small) Lourt., *Oxalis* y *Trifidus* Lourt. Bradea Vol. VII nº 2. Rio de Janeiro. Brasil.

O'Brien TP, McCully ME (1981). The study of plant structure. Principles and selected methods. Termarcarphi Pty. Ltd., Melbourne. Australia.

Raj B, El-Ghazaly G (1987). Morphology and taxonomic application of orbicules (Ubisch bodies) in Chloanthaceae. Pollen Spores 29: 151-166.

Rosenfeldt S, Galati BG (2007). Pollen morphology of *Oxalis* sps. from Buenos Aires province (Argentina). Biocell 31(1): 13-21.

Rowley JR, Walles B (1985). Cell differentiation in microsporangia of *Pinus sylvestris* III. Late pachytene. Nord J Bot 5: 255-271.

Rowley JR, Walles B (1987). Origin and structure of Ubisch bodies in *Pinus sylvestris*. Acta Soc Bot Pol. 56: 215-227.

Schols P, Furness CA, Wilkin P, Huysmans S, Smets E (2001). Morphology of pollen and orbicules in some *Discorea* species and its systematic implications. Bot J Linn Soc. 136: 295-311.

Ubisch G (1927). Zur Entewicklungsgeschichte der Antheren. Planta 3: 490-495.

Vinckier S, Smets E (2002a). Morphology, ultrastructure and typology of orbicules in family Loganiaceae *s.l.* and related genera, in relation to systematics. Rev Palaeobot Palynol. 119(3-4), 161-189.

Vinckier S, Smets E (2002b). Morphological and ultrastructural diversity of orbicules in relation to evolutionary tendencies in Apocynaceae *s.l.* Ann Bot. 90: 647-662.

Vinckier S, Smets E (2002c). Systematic importance in orbicule diversity in Gentianales. Grana 4: 158-182.

Vinckier S, Smets E (2003). Morphological and ultrastructural diversity of orbicules in Gentianaceae. Ann Bot. 92: 657-672.

Vinckier S, Huysmans S, Smets E (2000). Morphology and ultrastructure of orbicules in the subfamily Ixoroideae (Rubiaceae). Rev Palaeobot Palynol. 108: 151-174.